# Biological DE Nitrification of Wastewater with Immobilized Cells of *Pseudomonas stutzeri* attached to Polypropylene and Polyoxymethylene

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***Abstract*—**The study is aimed at investigating the effect of different biofilm carriers on immobilization of denitrifying bacteria for denitrification of wastewater using attached growth process. The carriers used in the study are polypropylene (low density) and polyoxymethylene (high density) for immobilizing *pseudomonas stutzeri.* The carriers with immobilized cells are then introduced to a fluidized bed bioreactor to remove nitrates from synthetic wastewater using methanol as carbon source. Experimental work has been carried out at optimum values of the parameters like airflow rate (2.5 lpm), temperature ($30^0$C), carbon source (85 mg/L), pH (7). The amount of carriers has been changed from 10 gm/L to 25 gm/L. It is noticed that the system is capable of removing nitrates from the wastewater. Polypropylene carrier has been found to be more efficient for nitrate removal with an optimal value of 15 gm/L compared to polyoxymethylene. The results indicate more than 90% nitrate removal using polypropylene carrier and 50 % using polyoxymethylene.

***Keywords*—**Biological denitrification, Fluidized bed bioreactor, Polypropylene, Polyoxymethylene, *Pseudomonas stutzeri.*

## I. Introduction

Removal of Nitrates is an important environmental issue as nitrate is one of the most common groundwater contaminants world-wide and discharge of nitrogen components into the environment can be a cause of serious problems such as eutrophication of rivers and deterioration of water sources, as well as a hazard for human and animal health and also to the environment. Denitrification is an important step in the nitrogen cycle in which nitrate ($NO_3$), is converted via nitrite ( $NO_2$ ) to nitric oxide (NO), to nitrous oxide($N_2O$) and nitrogen gas ($N_2$). It is the only biological mechanism by which $N_2$ is returned to the atmosphere. The process is carried out by a variety of bacterial species, which are found throughout natural environment. However, it has also been linked to stomach cancer [1-4], and blue baby syndrome [5-6]. Denitrifiers may play an important part in the breakdown of various hydrocarbon compounds. In agricultural areas denitrification leads to a loss of fertilizer effiency [7].

Biological denitrification is an attractive treatment option, in which the denitrifying bacteria converts nitrate to inert nitrogen gas and the waste product usually contains only biological solids. Biological removal of nitrate is widely used in the treatment of domestic and complex industrial wastewaters [8-15].

The application of cell immobilization techniques to the wastewater treatment process has recently gained much attention. The treatment of wastewater in fluidized bed bioreactor using immobilized cells is attracting increasing interest and has prompted the examination of different immobilization methods and a variety of carriers [16-22]. The denitrification could be achieved either in the suspended or attached growth systems. Immobilization by attachment can be obtained by spontaneous biomass adhesion onto porous support media and favored for denitrification of wastewater. Several natural materials (agar, agarose, collagen, alginates and chitosan) and synthetic polymer materials (poly acryl amide, polyurethane, polyethylene glycol and polyvinyl alcohol) have been applied as media [23]. Among the various matrixes that are available, the Polypropylene has been chosen for its ease of use, low cost, low toxicity, and high operational stability. Because of these features there is an increasing interest in the development of new fields of application. The polypropylene media provides a continuously high cell concentration in the bioreactor. To ensure complete denitrification, an external carbon source is often used that serves as the electron donor and facilitates the denitrification process [24-28].

## II. Materials And Methods

### *A. Inoculation and Cell immobilization*

Pure culture of *Pseudomonas stutzeri*, a denitrifying bacterium was sub-cultured once in a month and grown in the composition containing (per liter) 10 g of peptone, 10 g of beef extract, 5 g of Nacl and 20 g of agar-agar for the slant

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preparation. Polypropylene beads were used as the supporting media for immobilization of microorganism. The bacterium from the slants was inoculated into liquid broth (synthetic wastewater) containing poly propylene beads.

### *B. Synthetic wastewater*

The synthetic wastewater was prepared using deionized water in addition to other chemicals. Potassium nitrate was added as the nitrogen source at a concentration of 200-300 mg/L. Trace mineral constituents essential to the bacterial growth are added per liter of distilled water: $KNO_3$, 48.9 mg; $CH_3OH$, 85 mg; $MgSO_4.7H_2O$, 6 mg; $FeCl_3.7H_2O$, 0.2 mg; $Na_2HPO_4$, 430 mg; and $Na_2H_2PO_4$,320 mg. The composition gives the initial nitrate concentration of 30 mg/L and to increase or decrease the nitrate composition, the amount of potassium nitrate was varied proportionately. The experiments were conducted for 150 and 180 mg/L.

### *C. Analytical methods*

A sample of effluent was collected every 1 hour and after filtering through membranes, the concentration of nitrates was determined by Ion Potentiometer (Orion make) according to standard methods [29].

### *D. Experimental setup*

As shown in Fig. 1, the fluidized bed bio-reactor consists of a glass column of 0.5m height, 93 mm of Internal Diameter (ID) and 100mm of Outer Diameter (OD) with a capacity of 3.4 liters. The setup was provided with a glass jacket of 118 mm ID and 122 mm OD, to maintain the temperature of the reactor system at the set point and also provision was made for the supply of air. A gas sparger was located at the base of column for uniform distribution of gas.

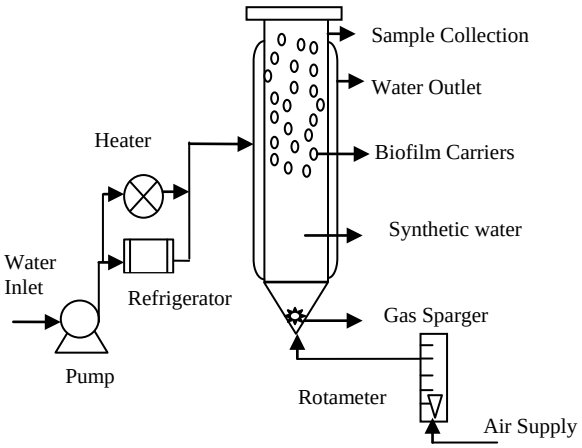


Fig.1 Fluidized Bed Bioreactor

## III. Results And Discussion

A fluidized bed bio reactor, with polypropylene beads as carrier and methanol as carbon source, was investigated for biological denitrification of wastewater at different initial nitrate concentration. Synthetic wastewater containing nitrate of 1 litre is taken into the reactor for biological treatment and air was fed from bottom with solids being fluidized at the top due to low density. The particle size and quantity is an important factor for the formation of a smooth fluidized bed. As the amount of polypropylene beads increases, the rate of biological dentrification decreases.

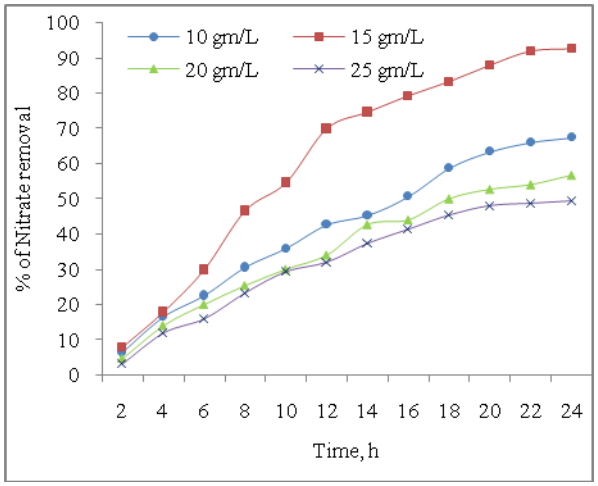


Fig.2 Percentage of nitrate removal at 150 mg/L using Poly propylene biofilm carriers

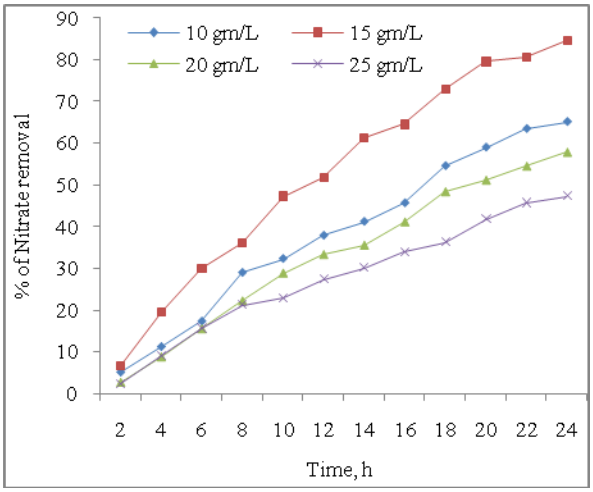


Fig.3 Percentage of nitrate removal at 180 mg/L using Poly propylene biofilm carriers

It is clearly seen from Figs. 2 and 3 that nitrate removal increases when the amount of Poly propylene beads increases from 10 gm/L to 15 gm/L and thereafter a decrease in nitrate removal is noticed with increase in the amount of beads. Due to low density of poly propylene, at low airflow rate the maximum amount of fluidization takes place and more removal was observed. At low airflow rate the destruction and decomposition of immobilized bacteria does not occur. From the above mentioned figures, the biomass concentration is also more at the same limits as compared with high amount beads of poly propylene.

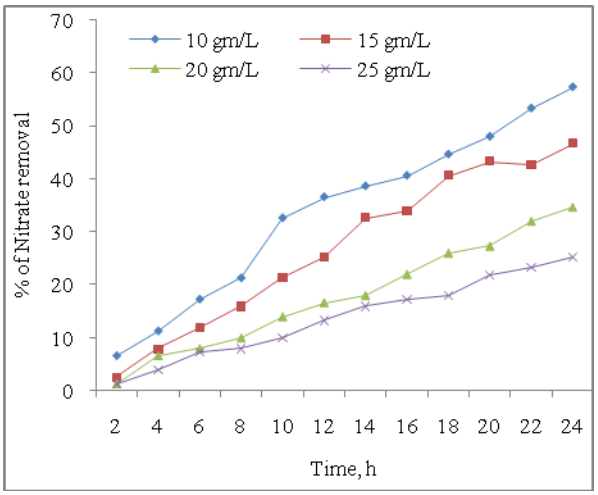


Fig.4 Percentage of nitrate removal at 150 mg/L using Polyoxymethylene biofilm carriers

From Figs. 4 and 5 representing denitrification using Polyoxymethylene, which is a high density polymer and the nitrate removal efficiency is less compared to low density polymer due to less fluidization. Increase in amount of beads for high density polymer requires higher air flow rate to fluidize the particle and also the chances of destruction and decomposition of immobilized bacteria were more. At 10 gm/L of high density polymer the removal efficiency of nitrate and biomass was more compared to high amount of polyoxymethylene. Therefore from all the figures it is seen that the removal efficiency of nitrate and biomass production is very less compared to low density polymer as carrier.

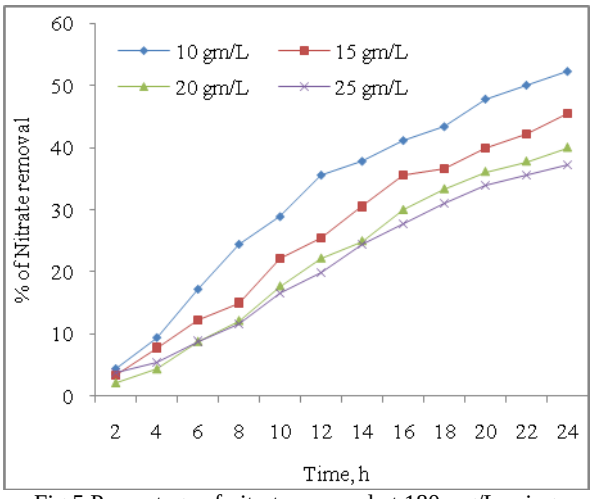


Fig.5 Percentage of nitrate removal at 180 mg/L using Polyoxymethylene biofilm carriers

## IV. Conclusions

Denitrification studies with attached growth biofilm on polypropylene and polyoxymethylene in a batch fluidized bed bioreactor system has been investigated as function of nitrate concentration and other environmental factors. The denitrification reactor design used in this study was effective at significantly reducing nitrate concentrations within a relatively short timeframe using *Pseudomonas Stutzeri*. As a result of this investigation, it was found that up to 180 mg/L feed nitrate concentration; the present system is able to produce an effluent with nitrate content below allowed limits with 24 h hydraulic retention time (HRT). The poly propylene beads presented the high percentage of nitrate removal throughout the experiment with 15 gm/L of biofilm carriers compare to polyoxymethylene. Experimentally, it was confirmed that the immobilized bacterium on polypropylene achieved higher than 90% of nitrate removal efficiency, whereas for polyoxymethylene the removal efficiency is nearly 50%.

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